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14. ABSTRACT

How Brca1 and p53 collaborate in tumorigenesis and how the Brca1 mutation affects breast cancer response to chemotherapeutics are not well understood. Mice carrying somatic mutations of Brca1 and p53 in the mammary epithelium cells that lead to accelerated tumor formation. The inactivation of Brca1 and p53 under a constitutive active MMTVCre or WAPCre results in complete penetrance of mammary tumors with a median tumor latency of 15.1 months and 6.6 months, respectively. There is a significant acceleration compared to the median tumor latency of 17.5 months and 10.5, respectively, in mice carrying mutated p53. Acceleration of tumorigenesis in the Brca1 and p53floxed mice is correlated with a rapid progression from hyperplastic to carcinoma. WAPCre targeted p53-deficient cells developed both ERb-positive and -negative tumors, while Brca1 mutation resulted in a greater frequency of ERb-negative tumors with high rates of lung and liver metastasis. The effects of doxorubicin and cisplatin treatment on spontaneous tumors and tumor transplantations were examined. Although the absence of Brca1 and p53 accelerate tumor growth, CDDP but not doxorubicin was effective in slowing tumor growth. Neither treatment was effective for tumors with a p53 deficiency.

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INTRODUCTION

BRCA1 and *BRCA2* are breast cancer susceptibility genes where mutations lead to cancer phenotypes that account for the hereditary breast and ovarian cancer syndrome[1-3]. Hereditary breast cancer accounts for 5-10% of all breast cancers diagnosed each year[4] and of these, *BRCA1* mutations are seen in 45-50% of these cases[5]. In sporadic breast and ovarian cancer, *BRCA1* mutations are rare[6-7]; however, 30-40% of sporadic cancers show reduced or absent expression of BRCA1[8]. *BRCA1* is a nuclear protein that contains multiple functional domains interacting with numerous molecules, which include products of tumor-suppressor genes, oncogenes, DNA damage-repair proteins, cell cycle regulators and transcriptional activators and repressors[9-10].

Using predictive biomarkers for response to DNA-damaging chemotherapy in breast and ovarian cancer has been investigated by looking at the DNA damage response gene *p53* and the results obtained from these studies have been contradictory[11-12]. Since many chemotherapeutic agents function by damaging DNA directly or indirectly, the role of *BRCA1* after chemotherapy-induced DNA damage and as a predictive biomarker of response to these drugs has been the subject of several *in vitro* and *in vivo* studies[9]. Given that one of BRCA1's functions is to help repair damaged DNA, *BRCA1*-disrupted mouse embryonic stem cells and mouse embryonic fibroblasts (MEFs) were more sensitive to the alkylating agent mitomycin C, the platinum compounds cisplatin, carboplatin and oxaliplatin and various topoisomerase I & II poisons[13-15]. Clinical trials that address the role of *BRCA1* in response to chemotherapy have been conducted[9]. Unfortunately, all trials have been retrospective and no trial was designed to study the role of *BRCA1* in response to chemotherapy. Therefore, to better study the role of BRCA1 in response to chemotherapy, we have generated mice carrying somatic mutations of *Brca1* and *p53* in the mammary epithelium cells which have lead to accelerated tumor formation.

KEY RESEARCH ACCOMPLISHMENTS

Here, we have established an animal model that closely mimics human BRCA1-mediated breast tumorigenesis. Our mouse model represents many of the same features seen in its human counterpart. For example, *BRCA1*-mutant breast cancers are characterized by high nuclear grade, *p53* mutation, ER_α and PR negativity and *myb* and *c-myc* amplification. Sporadic breast cancer cases often show amplification of ErbB-2 and overexpression of cyclin D1 and Bcl-2. However, in *BRCA1*-mutant breast tumors, the expression levels of ErbB-2, cyclin D1 and Bcl-2 are rarely amplified or overexpressed. We established the mouse model by using the Cre/loxP system in which mice, we previous established, carrying the floxed p53 gene were crossed to mice carrying the Brca1 floxed gene to generated, double floxed mice. In order to get deletion of p53 and Brca1 we then further crossed the mice to MMTV-Cre or WAP-Cre. After deletion of these genes, the rate of tumor formation was greatly enhance in double mutant mice (p53/Brca1) compared to that of single mutant mice (p53; Figure 1). While the tumor rate was ongoing, characterization of the tumors was carried and these tumors revealed many similar features to the one described above. Our tumors were highly ER_α and PR negative, HER2 negative and basal-cell like. The next step was to examine how these tumors responded to various chemotherapeutic agents. The agents chosen were doxorubicin, cisplatin and carboplatin (this was added later because it is less toxic to patients and the animals). In vitro data comparing p53 tumor to p53/Brca1 tumors showed no difference between the different chemotherapeutic agents. Reasons for this are still ongoing. However, the in vivo studies carried using spontaneous or transplanted tumors showed different results. Although the absence of Brca1 and p53 accelerate tumor growth, cisplatin and carboplatin but not doxorubicin was effective in slowing tumor growth. Neither treatment was effective for tumors with a p53 deficiency. Currently, why these tumors are so responsive to cisplatin and carboplatin but not doxorubicin are being investigated along with reasons why we see an accelerated tumor formation in p53/Brca1 mutant mice. In

completing the in vivo studies, we noticed that after a few months, the tumors began to reoccur. Once this occurred, we confirmed the results and we have established a cisplatin-resistance model for Brca1 mediated tumorigenesis. Resistance always occurs in patients and to find a way around this will provide a treatment outcome. We plan to identify gene involved in cisplatin resistance through array CGH or DNA microarray. Once we have identified possible gene, we will study them in more detail and try t identify potential small molecules that can inhibit these genes.

REPORTABLE OUTCOMES

Currently, we are in the process of understanding why Brca1-mediated tumors are so responsive to cisplatin. Once understand, the manuscript will be sent out for publication.

CONCLUSIONS

This study here took the approach of pharmacogenetics by looking at what mutations are found then applying the correct chemotherapeutic agent to treat these tumors. We know the function of Brca1 therefore, we examined a common chemotherapeutic agent, doxorubicin and one that targets the DNA double strand break repair protein, cisplatin and carboplatin. Our results indicate that if you understand which mutations the patients then a more favorable outcome can occur. We also hope in the future to provide some new insights into why are patients resistance to chemotherapeutics agents like cisplatin.

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APPENDICES

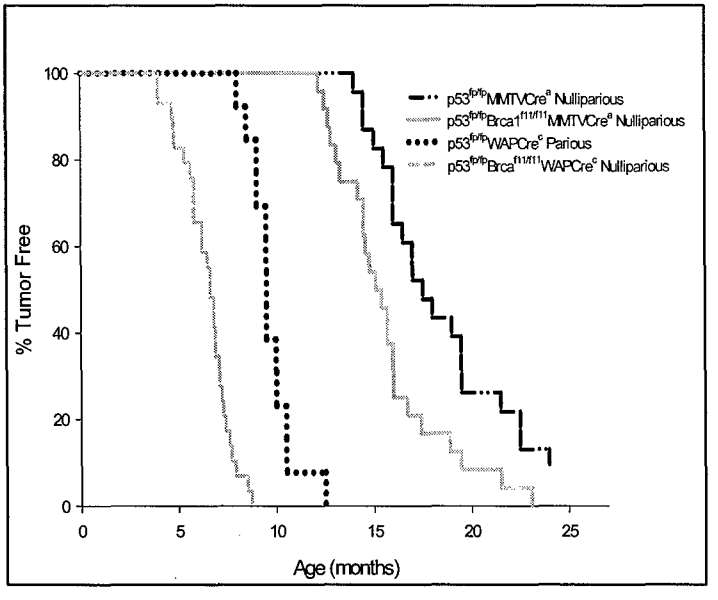

Figure 1- Kaplan Meier plot comparing p53 mutated mice (black) to p53/Brca1 mutated mice (gray). This shows the accelerated rate of tumorigenesis in the p53/Brca1 mice.

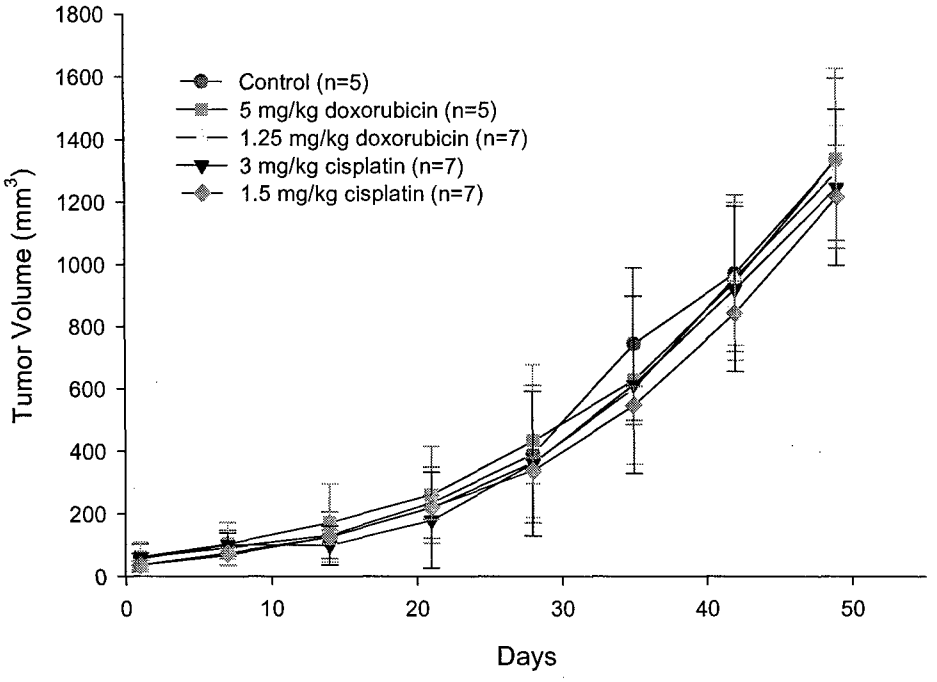

Figure 2- **Lack of response of *p53* tumors to Doxorubicin & CDDP *in vivo.***

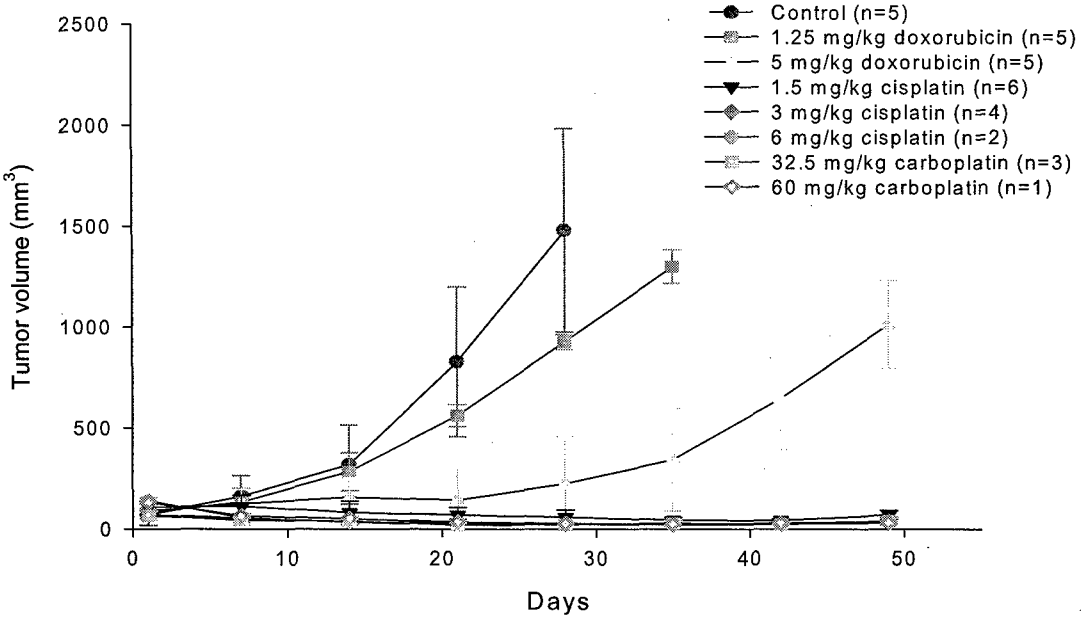

Figure 3- **Response of *p53Δ5&6Brca1Δ11* mammary tumors to doxorubicin, CDDP or carboplatin *in vivo***